

WHAT IS CLAIMED IS:

1. A stable formulation of Apo-2 ligand, comprising Apo-2 ligand and about 0.2M to about 0.5M salt, wherein said formulation has a pH of about 6 to about 9.

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2. The formulation of claim 1 wherein said salt is arginine salt.

10 3. The formulation of claim 2 wherein the concentration of said arginine salt in the formulation is about 0.4M to about 0.5 M.

15 4. The formulation of claim 2 wherein the arginine salt is selected from the group consisting of arginine succinate, arginine sulphate, arginine malate, arginine citrate, arginine tartrate, and arginine phosphate.

5. The formulation of claim 2 wherein the arginine salt is arginine succinate.

20 6. The formulation of claim 1 wherein the salt is sodium sulphate.

7. The formulation of claim 1 or 6 wherein the Apo-2 ligand comprises crystallized protein.

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8. The formulation of claim 1 wherein said formulation is lyophilized.

30 9. The formulation of claim 1 wherein the pH of said formulation is about 6.5 to about 8.5.

10. The formulation of claim 9 wherein the pH of said formulation is about 7 to about 7.5.

35 11. The formulation of claim 1 wherein the concentration of Apo-2 ligand is about 1 mg/ml to about 20 mg/ml.

12. The formulation of claim 1 wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1.

13. The formulation of claim 12 wherein said Apo-2 ligand is not linked or fused to an epitope tag.

5 14. The formulation of claim 1 wherein said formulation further comprises surfactant.

15. The formulation of claim 14 wherein said surfactant is a polysorbate or poloxamer.

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16. The formulation of claim 14 wherein the concentration of said surfactant in the formulation is about 0.005% to about 0.2%.

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17. The formulation of claim 1 wherein said formulation further comprises buffer.

18. The formulation of claim 17 wherein said buffer is Tris buffer.

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19. The formulation of claim 18 wherein the pH of the formulation is about 7 to about 7.5.

20. The formulation of claim 1 wherein said formulation further comprises one or more divalent metal ions.

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21. The formulation of claim 20 wherein said one or more divalent metal ions is zinc.

22. The formulation of claim 1 further comprising a preservative.

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23. The formulation of claim 1 wherein said formulation is storage-stable for at least 12 months.

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24. The formulation of claim 23 wherein said formulation is storage-stable for at least 24 months.

25. A stable, lyophilized formulation of Apo-2 ligand, comprising about 1 mg/ml to about 20 mg/ml Apo-2 ligand, about 0.2 M to about 0.5M arginine salt, buffer, and surfactant, wherein said

formulation has a pH of about 6 to about 9.

26. The formulation of claim 25, wherein said arginine salt is arginine succinate.

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27. The formulation of claim 26, wherein the concentration of said arginine succinate is about 0.4M to about 0.5M.

28. The formulation of claim 25, wherein said buffer is Tris  
10 buffer.

29. The formulation of claim 25, wherein said surfactant is a polysorbate.

15 30. The formulation of claim 25, wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1.

31. The formulation of claim 25, wherein said formulation further comprises one or more divalent metal ions. <sup>6</sup>

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32. A stable formulation of Apo-2 ligand, comprising about 1mg/ml to about 20 mg/ml Apo-2 ligand, about 0.2M to about 0.5 M salt, buffer, and surfactant, wherein said Apo-2 ligand comprises crystallized protein and said formulation has a pH of about 6 to  
25 about 9.

33. The formulation of claim 32, wherein said salt is sodium sulphate.

30 34. The formulation of claim 32, wherein said buffer is Tris buffer.

35 35. The formulation of claim 32, wherein said surfactant is polysorbate.

36. The formulation of claim 32, wherein said formulation has a pH of about 7 to about 7.5.

37. A stable formulation of Apo-2 ligand, comprising about 0.1

mg/ml to about 2 mg/ml Apo-2 ligand, sugar, and surfactant, wherein said formulation has a pH of about 6 to about 9.

38. The formulation of claim 37 wherein said sugar is trehalose.

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39. The formulation of claim 37 wherein the concentration of the sugar in the formulation is about 1% to about 8%.

40. The formulation of claim 37 wherein said formulation is  
10 lyophilized.

41. A method of making a stable formulation of Apo-2 ligand, comprising steps of (a) providing about 1 mg/ml to about 20 mg/ml Apo-2 ligand, about 0.2 M to about 0.5M arginine salt, buffer, and  
15 surfactant, (b) combining or mixing the ingredients of step (a) to make a formulation, and (c) adjusting the pH of the formulation of step (b) to about 6 to about 9.

42. The method of claim 41, wherein said arginine salt is  
20 arginine succinate.

43. The method of claim 42, wherein the concentration of said arginine succinate is about 0.4M to about 0.5M.

25 44. The method of claim 41, wherein said buffer is Tris buffer.

45. The method of claim 41, wherein said surfactant is a polysorbate.

30 46. The method of claim 41, wherein said Apo-2 ligand comprises amino acids 114 to 281 of Figure 1.

47. A method of making crystallized Apo-2 ligand, comprising steps of (a) providing Apo-2 ligand, buffer, and monovalent  
35 cationic salt, (b) combining or mixing the ingredients of step (a) to make a formulation at a temperature of about 20° C to about 30° C, and (c) lowering the temperature of the formulation of step (b) to about 2° C to about 8° C; wherein Apo-2 ligand crystallization occurs as the temperature of the formulation of step (b) is

lowered.

48. The method of claim 47, wherein said salt is sodium sulphate or sodium chloride.

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49. The method of claim 48 wherein the concentration of the salt is 0.1M to about 0.15M.

50. The method of claim 47, wherein the formulation of step (b)  
10 is agitated as the temperature is lowered in step (c).

51. The method of claim 47, wherein the method further comprises a step (d) in which the Apo-2 ligand crystals are dried.

15 52. The method of claim 51, wherein prior to said step (d), the Apo-2 ligand crystals are washed.

53. A method of making Apo-2 ligand, comprising the steps of: (a) providing host cells comprising a vector containing DNA encoding  
20 Apo-2 ligand; (b) culturing the host cells in culture medium under conditions sufficient to express Apo-2 ligand; (c) obtaining said expressed Apo-2 ligand from the host cells and culture medium; (d) formulating said Apo-2 ligand into a solution containing sodium chloride or sodium sulphate to make a formulation at a temperature  
25 of about 20° C to about 30° C, and (e) lowering the temperature of said formulation of step (d) to about 2° C to about 8° C, wherein Apo-2 ligand crystals form when the temperature of step (e) is lowered.

30 54. The method of claim 53 wherein prior to said step (d), the Apo-2 ligand protein is concentrated.

55. The method of claim 54 wherein the Apo-2 ligand protein is concentrated by centrifugation, column chromatography or  
35 ultrafiltration.

56. The method of claim 53 wherein step (d) is conducting by applying the Apo-2 ligand to a chromatographic column and eluting the Apo-2 ligand into a sodium chloride or sodium sulphate

containing buffer solution.

57. The method of claim 56 wherein said chromatographic column is a cation exchange column.

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58. The method of claim 56 wherein said cation exchange column comprises SP-Sepharose fast flow, CM-Sepharose fast flow, or Macro-prep ceramic HS.

10 59. The method of claim 56 wherein said buffer solution contains 50 mM Hepes, 50 mM Tris, 50 mM triethanolamine, 0.05% Triton X 100, 1 mM DTT, pH 7.5 - 8.0.

15 60. The method of claim 53 wherein the formulation is agitated during step (e).

61. The method of claim 53 wherein the pH of the formulation in step (d) is about 6.5 to about 8.5.

20 62. The method of claim 53 wherein said host cells are prokaryote cells.

63. The method of claim 62 wherein said prokaryote cells are *E. coli*.

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64. A device for administering a formulation of Apo-2 ligand to a mammal, comprising a container holding at least one dosage unit of the Apo-2 ligand formulation of claim 1, 25, 32, or 37.

30 65. The device of claim 64 wherein said device is a pen injector device.

66. The device of claim 64 wherein the container is a cartridge.

35 67. An article of manufacture, comprising a container which includes the Apo2L/TRAIL formulation of claim 1, 25, 32, or 37, and printed instructions for use of said Apo-2L/TRAIL formulation.

68. The article of manufacture of claim 67 where said container

is a bottle, vial, syringe, or test tube.

69. The article of manufacture of claim 67 which comprises a second container which includes water-for-injection, saline, 5 Ringer's solution, or dextrose solution.

70. A method of inducing apoptosis in mammalian cells, comprising exposing mammalian cells to an effective amount of the Apo-2 ligand formulation of claim 1, 25, 32, or 37.

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71. The method of claim 70 wherein said mammalian cells are cancer cells.

72. A method of treating cancer in a mammal, comprising 15 administering to a mammal diagnosed as having cancer an effective amount of the Apo-2 ligand formulation of claim 1, 25, 32, or 37.